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IN-SITU DECONTAMINATION OF
METAL-POLLUTED SOILS BY
METAL-ACCUMULATOR PLANTS

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4 pages
3 tables

1. Field Experiment Background

The Woburn Market Garden Experiment contains well-characterized plots known to be contaminated with heavy metals.

This contaminated soil was used to compare the extraction efficiencies of several plant species known as "hyperaccumulators". A range of these species, including a non-accumulating control, were grown up in a controlled environment before being planted out in April-May 1993.

Two species of *Thlaspi* (*T. caerulescens*, *T. ochroleucum*), three species of *Alyssum* (*A. murale*, *A. tenium*, *A. lesbiacum*), two genotypes of *Cardaminopsis halleri* (from France and Germany), and for comparison the control non-accumulating species (*Raphanus sativus*) were grown in plot 39, which is the most contaminated in this field experiment.

In previous years, *Cardaminopsis halleri* (seed from France) was grown on nine plots and almost all of them overwintered and were still present in 1993.

2. Experimental - Field Experiment

Every subplot was regularly visited, watered and weeded. Applications of a basal fertilizer solution were made on the 28 May and 10 August. This

supplied 150 kg N ha⁻¹ and 75 kg P and K ha⁻¹.

By the end of June, the radish plants were considered to have reached maturity and were therefore harvested on the 1 and 7 July 1993.

Roots and leaves were separately and carefully washed, the fresh weight measured and the material then oven dried at 80°C for 16 h.

After taking the dry weights, samples were ground on a "Christy and Norris" grinding mill. This procedure was followed for harvesting all the plants during the experiment.

During July, some regrowth of previous years *T. caerulescens* plants had been noticed in plots 22 and 31. Their seeds were therefore collected on the 6 August to be used for further experiments.

On the same date, the second season *Cardaminopsis halleri* plants covering half of the area in every sub-plot were harvested.

After being separated from the chaff by a blower, the seeds were labelled and stored at a temperature of 4°C.

All the dried and ground material was digested using a mixture of concentrated nitric and perchloric acids.

The metal concentrations in the digests were determined using a ARL 34000 inductively-coupled plasma emission spectrometer (ICP).

The results of this digestion will be presented in the final report.

This year was cold and wet and the growth and development of the plants was retarded by this. It seemed likely that the *Abyssum* and *Thlaspi* plants need to overwinter before they can reach maximum biomass. The decision was taken, therefore, to harvest these next summer.

3. Results - Field Experiments

The recorded fresh weight, dry weight and harvested area of the *C. halleri* which survived in sufficient quantity to be harvested are shown in Table 1. The calculated yields in t/ha varied across the plots from 1.73 - 5.5 t because of variation in the numbers of *C. halleri* plants which survived the winter. The concentrations of Zn also varied, from 1313 to 3584 mg Zn/kg dry matter. The concentrations of Zn, Ni and Cd in the plants did not relate to the increasing concentrations of these metals in the test soils (Table 2). The greatest influence on the concentrations and particularly the removal of these metals was yield; plots 31, 36 and 33 had lower yields and the smallest removals (Table 1). Because of the variation in yield and concentration of Zn, the calculated total Zn removal varied from 2.4 - 18 kg Zn/ha, and Cd from 14-82 g/ha.

In comparison, the radish produced about 5 times the dry matter yield, but only a tenth of the Zn removal of *C. halleri* (Table 3). Both species extracted roughly similar amounts of Cd, but radish extracted more Ni than the Zn accumulator plant. However, the total amount of Ni removed was not large (Table 3).

4. Technical Problems Encountered

Due to an outbreak of two fungal diseases it was necessary to treat the plots twice with an appropriate fungicide during the summer. The onset of disease was favoured by very humid conditions from April onwards.

The same conditions also caused some difficulties in establishment of some of the plants, especially among the *Cardaminopsis halleri*. The affected ones were replaced until the end of June.

As the same weather conditions persisted throughout the summer, it proved impossible to replace all the affected plants.

5. Glasshouse Experiment

Not all the hyperaccumulator genotypes are capable of accumulating heavy metals to the same great extent.

In order to assess the performance of different genotypes and different species to remove toxic metals from contaminated soils, a new experiment was set up in the glasshouse.

The efficiency of removal of metals and biomass production will be tested.

By collecting the seeds of the plants which grow well and remove the greatest quantities of heavy metals from the soil it is hoped to select for individuals with increased metal extraction capability.

5. Experimental - Glasshouse Experiment

The soil available for this experiment was very contaminated and so it was mixed with 50% loam to dilute the metal concentrations.

About 60 kg of this mixed soil was dried and sieved ($< \frac{1}{4}$ ") before being subjected to Aqua Regia digestion and metal analysis.

Seeds from seven different genotypes of *T. caerulescens*, two of *C. halleri* and only one of *T. ochroleucum* were sown in sterile conditions. Seeds were placed on water agar for a period of about ten days and the seedlings then planted out in 3 cm pots in the glasshouse.

The selected genotypes are listed below.

Thlaspi caerulescens:

- a) 00007e Cumbria, UK
- b) 00012 Belgium
- c) 00015e Derbys, UK
- d) 00016 Cumbria, UK
- e) 00095g Derbys, UK
- f) 00096a Derbys, UK
- g) 00099 NWales

Thlaspi ochrolecum:

- h) Thasos, Greece

Cardaminopsis halleri:

- l) 00022b Lille, France
- m) Lille, France

Up to eighty pots per genotype were put into plastic trays and watered regularly. Age, dimensions, shape and other phenotypic features of every plant were recorded. A fertilizer solution was applied every two weeks to supply 50 mg K and N per pot. At the beginning of September, most plants were ready to be analysed.

Three leaves were taken from every plant; the leaves were carefully washed, weighed and oven dried at 80°C overnight. The samples were

then digested in nitric-perchloric acid and analysed by means of a ICP spectrometer.

The results will be presented in the final report.

6. Technical Problems Encountered

Mixing the limited amount of contaminated soil with the loam increased the volume of soil available. As a large number of pots were required, a small size was used. The small pots tend to loose moisture rapidly resulting in the soil fusing together as a solid mass. To overcome this the soil was kept moist, however this encouraged fungal pathogens.

To counteract the moisture excess, all pots were placed on a layer of sand. Later, a totally new artificial soil was created by spiking a standard loam with the metals cadmium, zinc and nickel.

7. Future Work

Further analysis of the year's results will be performed before writing the final report.

The remaining plants in the field experiment will be harvested and analysed in the summer of 1994.

Table 1. Yields, metal concentrations and metal removal in *Cardaminopsis halleri*, 1993.

Plot	Fresh wt (g)	Dry wt (g)	Area (cm ²)	Yield (t DM/ha)	Zn (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	Zn (kg/ha)	Ni (g/ha)	Cd (g/ha)
31	283	40	2100	1.90	1313	2.21	8.97	2.49	4.20	17.04
36	415	53	3055	1.73	1380	2.55	12.51	2.39	4.41	21.64
38	364	55	1000	5.50	2396	2.22	8.47	13.18	12.21	46.59
22	460	95	1875	5.07	3584	2.76	16.3	18.17	13.99	82.64
33	331	45	2450	1.84	1501	3.16	7.86	2.76	5.81	14.46
16	721	106	3250	3.26	3293	3.25	9.24	10.74	10.60	30.12
39	617	96	3104	3.09	3264	4.13	11.68	10.09	12.76	36.09
Mean	456	70	2404	3.20	2390	2.90	10.72	8.55	9.14	35.51

Details:

Calculation of yield

$$\text{cm}^2 / 10,000 = \text{m}^2$$

$$\text{m}^2 \times 10,000 = \text{ha}$$

$$\text{g/ha} / 1000 = \text{kg/ha}$$

$$\text{i.e. DM (g) / area (cm}^2\text{) } \times 100 = \text{t/ha}$$

Calculation of removal

$$\text{mg/ha} / 1000 = \text{kg/ha}$$

$$\text{i.e. dm(t/ha) } \times \text{conc (mg/kg) } = \text{g/ha}$$

$$\text{i.e. dm (t/ha) } \times \text{conc (mg/kg) } / 1000 = \text{kg/ha}$$

Table 2. Concentrations of metals in test plots in 1992

Plot	Zn (mg/kg)	Ni (mg/kg)	Cd (mg/kg)
31	125	20.3	2.25
36	169	23.8	4.19
38	224	28.5	5.40
22	253	31.6	5.86
33	273	31.8	6.39
16	383	33.7	7.52
39	408	34.5	8.55

Table 3. Yields, metal concentrations and metal removal in Radish, 1993.

	Fresh wt (g)	Dry wt (g)	Area (cm ²)	Yield (t DM /ha)	Zn (mg/ kg)	Ni (mg/ kg)	Cd (mg/ kg)	Zn (kg /ha)	Ni (g /ha)	Cd (g /ha)
Leaves + stems	3173	537	4444	12.08	69.18	2.72	1.49	0.84	32.86	18.00
Roots (hypocotyl)	1650	139	4444	3.13	112.2	3.11	1.28	0.35	9.73	4.01
Total	4823	676	4444	15.21	78.03	2.80	1.44	1.19	42.59	21.90